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DISPARATE EFFICACY OF COLLAGEN HYDROLYSATE AND GLUCOSAMINE ON THE EXTRACELLULAR MATRIX METABOLISM OF ARTICULAR CHONDROCYTES

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Purpose: Glucosamine has been used in the treatment of osteoarthritis (OA) for several years. More recently, collagen fragments were shown to provide a positive effect on joint health. The objective of this study was to investigate the efficacy of a specific type of collagen hydrolysate (CH) on the biosynthesis of ECM macromolecules in comparison with glucosamine sulfate (GS) and glucosamine hydrochloride (GH) in a chondrocyte culture model.
Methods: Primary porcine articular chondrocytes and human femoral head chondrocytes were cultured under reduced oxygen conditions. The culture medium was supplemented with various concentrations of CH. In parallel experiments chondrocytes were treated with either GS or GH in various concentrations up to 2.5mM. At the end of the culture period the amount of cell-associated proteoglycans (PGs) and the extent of PG synthesis were quantified by measuring ^{35}S-sulfate incorporation and by colorimetric assay. The amount of aggrecan accumulated in the ECM was determined by Western Blotting. In addition, type II collagen biosynthesis was quantified by means of ELISA technique. The results were confirmed by immunocytochemical detection of type II collagen and by analyzing the incorporation of ^{14}C-proline into matrix proteins.
Results: Supplementation of the culture medium with CH resulted in a statistically significant ($p<0.05$) increase of PG synthesis. The amount of cell-associated PGs was almost doubled after CH treatment, compared with the control cells. Administration of CH was also associated with increased aggrecan expression and a statistically significant ($p<0.05$) 1.5-fold increase of type II collagen biosynthesis. In contrast, the administration of GS or GH had no stimulatory effect on the type II collagen biosynthesis of the chondrocytes. Moreover, although slight differences could be observed between GS and GH, supplementation of glucosamine had no significant effect on the amount of cell-associated PGs, or total PG synthesis, compared to the controls.
Conclusions: These results indicate a stimulatory effect of CH on the synthesis of PG and type II collagen. In contrast, GS and GH failed to stimulate the synthesis extracellular matrix (ECM) macromolecules by chondrocytes.
These data suggest that CH may help reduce degenerative changes of the ECM by stimulating anabolic processes in cartilage tissue.

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TGF-β1 PROTECTS OA, BUT NOT NORMAL, HUMAN CHONDROCYTES FROM Ro 31-8220 AND TNF-α INDUCED APOPTOSIS

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Purpose: Death of chondrocyte cells by apoptosis is a hallmark of degenerative joint diseases such as osteoarthritis (OA). Combination of Tumour Necrosis Factor α (TNF-α) and Ro 31-8220 (Ro) have been proved to induce apoptosis in chondrocytes. Transforming Growth Factor β-1 (TGF-β1) is a pleiotropic cytokine that provides signals for both cell survival and apoptosis, the final output depending on cell type and cellular context.
Objectives: In this work, we studied if TGF-β1 is able to protect human chondrocytes from apoptosis induced by an *in vitro* model (TNF-α + Ro), both in OA and normal chondrocytes.
Methods: Human OA and N cartilage was obtained from the femoral heads of 8 patients each. OA cartilage was obtained from patients who were undergoing joint replacement while normal cartilage was obtained from cadavers who had no history of joint disease and who had macroscopically normal cartilage. Apoptosis was asessed by flow cytometry (propidium iodide) and ELISA cell death, while nuclear morphology was evaluated using the fluorescent stain DAPI (4',6-diamidino-2-phenylindole, dihydrochloride).
Results: It was established two groups of cells, one group was preincubated for 120h with TGF-β1, while the another group was not, both in OA cells and normal cells. Afterwards, both groups were stimulated with TNF- α and Ro for 16h. In OA cells, TGF-β1 significantly reduced the percentage of hypodiploid chondrocytes (TNF-α+Ro 18.6% vs TGF-β1+ TNF-α+Ro 8.9%, $p< 0.05$), just as the percentage of internucleosomal DNA breakage (TGF-β1+ TNF-α+Ro 40.3% vs TNF-α+Ro 100%; $p< 0.05$), as a result of apoptotic induction. However, in normal chondrocytes, TGF-β1 did not induce protection against apoptosis as we asses both the percentage of hypodiploid chondrocytes (TNF-α+Ro 20.3% vs TGF-β1+ TNF-α+Ro 25.2%, $p= 0.381$) and internucleosomal DNA breakage (TGF-β1+ TNF-α+Ro 83.0% vs TNF-α+Ro 100% $p= 0.391$). Furthermore, nuclear morphology using DAPI fits well with previous results, both in OA and normal chondrocytes.
Conclusions: These results show that TGF-β1 is able to partially blocks the apoptosis induced by TNF-α+Ro 31-8220 in OA chondrocytes, but not in normal chondrocytes. In some way, this result emulate the pleiotropic behaviour of TGF-β1 *in vivo*, providing a model to study the differences at signal transduction triggered by TGF- β1 in OA and normal chondrocytes.

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DERMATAN SULFATE REMOVAL DOES NOT CHANGE INDENTATION PROPERTIES OF ARTICULAR CARTILAGE

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Purpose: Decorin is attached to the Type II collagen fibrils in articular cartilage. It has been hypothesized that the dermatan sulfate (DS) attached to the decorin core protein projects out from the protein and forms a bond with adjacent fibrils, either directly to another DS chain or through an intermediary molecule, thus serving as a gluing molecule between the collagen molecules. As such, decorin would serve as a structural molecule, carry load in cartilage, and its damage would lead to change in mechanical properties. The purpose of this study was to to test this hypothesis by digesting DS in bovine patellar cartilage with chondroitinase B (cB) and testing the cartilage by indentation before and after digestion.
Methods: Articular cartilage from bovine patella was tested in indentation before and after digesting with cB. For comparison, to validate our methods, we also tested cartilage before and after digestion with chondroitinase ACI (cAC), an agent known to alter indentation properties. In both cases, controls were cartilage specimens treated in the same way in buffer, without the enzyme. Digestion was with 0.1U/ml for 24 hrs at 37 ^{0}F with gentle agitation. Indentation was with a 2 mm diameter flat ended non-porous cylinder on an ELF 3100 (Bose, Inc., Minneapolis, MN). Indentation was with a series of 7 step-hold displacements. Step loads were applied in approximately 0.1 sec; hold times were 300 sec. Instantaneous modulus, equilibrium modulus, and relaxation function were determined for each test. 5 indents for